ROBUST SUMMARIES: Higher Alkenyl Succinic Anhydrides

1.0 **Biodegradation**

Test Substance	CAS #27859-58-1
Chemical Name	Butanedioic acid, (tetrapropenyl)
Remarks	Test material purity not provided
Method Method/Guideline followed Test Type (aerobic/anaerobic)Aerobic GLP (Y/N) Contact time (units) Inoculum	 OECD 301F Manometric Respirometry Test (Biodegradation) Y 28 days Activated sludge from domestic wastewater treatment plant.
Year (Study Performed)	1999
Remarks for test conditions Test System Inoculum Concentration of test chemical (assay conducted in duplicate reactor flasks) Dosing procedure	 The test system was a defined mineral medium inoculated with the supernatant of homogenized activated return sludge from a public wastewater treatment plant. The mineral medium was prepared as outlined in OECD Guideline 301F The supernatant from homogenized activated sludge was used as inoculum. A two-liter flask containing 100 mL of supplemented sludge supernatant and 900 mL of test medium was prepared. The inoculum was pre-adapted to the test material for 14 days during which the test substance was added incrementally at concentrations equivalent to 4, 8, and 8 mg carbon/L on days 0, 7, and 11, respectively. The targeted microbial level in the test mixture was 10,000 to 1,000,000 cells/mL. The actual microbial level in the test mixture was 1000 cells/mL. This deviation from the protocol was not considered significant. Test substance concentrations were 107.2 and 110.2 mg/L, giving a 122.1 and 125.5 mg ThOD. No organic solvents were used to facilitate the dispersion of the test material. The test substance was weighed onto a Teflon coupon and introduced into the medium. Test mixtures were stirred throughout the study using magnetic stirrers. Temperature of incubation: 23 +1 ºC A measured volume of the inoculated mineral medium containing

	approximately 107-110 mg/L test substance was continuously stirred in a closed system for 28 days.
Sampling frequency	The oxygen uptake was monitored continuously and recorded every 4 hours throughout the test.
Controls	Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Sodium benzoate was used as the positive control.
Analytical method	Oxygen uptake was measured using a BI-1000 electrolytic respirometer system. The hydrogen, nitrogen and total organic carbon content of the test material were determined.
Method of calculating measured concentrations	Material concentrations were not measured
Results Test Validity	All test validity criteria were met as follows: The average oxygen uptake of each of the two inoculum blanks was lower than 60 mg/L in 28 days. The difference in biodegradation levels of the reference and test substance replicates was less than 20%. The percent degradation of the reference material reached the pass level (60%) within 14 days. The final pH of the test mixtures were within the range of 6.0-8.5 demonstrating the biodegradation was not inhibited by extreme pH
Degradation: %after time	Test substance: 18.3% after 28 days Positive reference (sodium benzoate): =/>60% (3d)
Remarks Conclusion	18.3% after 28 days. The reference substance, sodium benzoate, reached a level of 94.2%in the same test period.

Data Quality	(1) Reliable without restriction
References	Unpublished Confidential Business Information taken from ACC HERTG panel Robust Summary "Alkenyl Succinic Anhydrides", Update Nov. 22, 2002
Other	

2.0 Ecotoxicity Category: Alkenyl Succinic Anhydride

AQUATIC ORGANISMS

2.3 Acute Toxicity to Aquatic Plants (e.g algae)

Test Substance	CAS #27859-58-1
Chemical Name	Butanedioic acid,(tetrapropenyl)-
Remarks	Test material purity not provided
Method Method /Guideline followed Test Type GLP (Y/N)	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1993), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984). Static acute toxicity test Y
Year (Study Performed)	1996
Species/Strain	Freshwater algae**,** *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*
Element basis (#of cells/mL)	Approximately 10,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	No
Statistical methods	Average specific growth rate was calculated as the natural log of the number of cells/ml at 72 and 96 hours minus the natural log of the number of cells/ml at 0 hour, divided by the hour of exposure. Results were interpreted by standard statistical techniques. All calculations were performed using nominal concentrations of the test material with the number of cells/mL, then with the average specific growth rates.
Remarks field for test conditions (fill as applicable) Test Species Test System	 Cells taken from in-house culture *Pseudokirchneriella subcapitata* originally purchased from the University of Texas at Austin alga collection. Each WAF was prepared only at the beginning of the test. A measured weight of test material was added to a measured volume of dilution water in a glass vessel and stirred for 20 hours. Stirring accomplished using a magnetic stirrer. Mixing speed was adjusted such that a vortex formed approximately 25% of the distance to the bottom. Following the mixing period, the test solution was allowed to stand for 4 hour before the water phase was removed. The siphoned water phase (i.e. WAF) was used

	for the aquatic toxicity test.
Test Conditions	A static test was conducted; i.e. there was no daily renewal of test solution. Two 100-mL replicates per treatment, inoculum ~10,000 cells/mL. The 250-mL Erlenmeyer flasks were covered to reduce entry of dust. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). The occurrence of relative size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers or aggregation of cells was determined visually by means of direct microscopic examination with a hemocytometer. Cell counts were made at 72 and 96 hours
Light	Cool-white fluorescent lights provided a light intensity of approximately 400-430 foot-candles.
Test temperature	24.0 C
Dilution Water	Sterile enriched alga growth media (US EPA, 1978) adjusted to pH 7.5. Measured total suspended solids in fresh untreated alga media were <10 mg/L, respectively. Test media pH was 7.4 at 0-hour and 10.2 after 96 hours.
Test Levels	Control and 0.3, 3.0, 33, 330 and 3300 mg/L WAF loading rates. Insoluble material was observed at 24, 48 and 96 hours in test vessels containing 330 and 3300 mg/L. No other insoluble material was observed during the study
Method of calculating mean measured concentrations	not applicable
Exposure period	96 hours
Analytical monitoring	Not performed concentrations were all based on nominal.
Results	96-h E_{C50} 93 mg/L; The 96-hr NOEC =33 mg/L.
Remarks	Insoluble material was observed at 24, 48 and 96 hours in test vessels containing 330 and 3300 mg/L; other insoluble material was observed during the study. The algal population grew well resulting in an average of 1,508,000 cells/mL in the control after 96 hours. Water quality was acceptable throughout the study. The two highest concentrations of test material significantly decreased the pH of the test media at the beginning of the test (330 mg/L pH: 4.3-4.4; 3000 mg/L pH: 3.9-4.0. No biological effects were noted during the study on cell size, shape, color, flocculation, adherence to test containers or aggregation.
Findings	

	The 96-hour no observed effect concentration (NOEC) was 33 mg/L. The calculated EC50s were as follows: Based on Number of Cells/mL 72 hr EC50: 100 mg/L (95%confidence interval 33-330 mg/L) 96 hr EC50: 93 mg/L (95%confidence interval 33-330 mg/L) Based on Average Specific Growth Rate 72 hr EC50: 100 mg/L (95%confidence interval 33-330 mg/L) 96 hr EC50:100 mg/L (95%confidence interval 33-330 mg/L) •The toxic effects were determined to be algistatic based on the rapid re-growth of an aliquot of cells taken from the 330mg/L test vessel and cultured in fresh control media
Test Validity	Control response was satisfactory.
Conclusions	The test material was considered algistatic to freshwater alga at loading rates of 330 and 3000 mg/L. 96-h E_{C50} 93 mg/L; The 96-hr NOEC =33 mg/L.

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Data Quality (1) Reliable with restriction. Restriction due to the lack of any analytical confirmation of test material concentration in test solutions. All concentrations are expressed as nominal.

References Confidential business information, taken from ACC HERTG panel Robust Summary "Alkenyl Succinic Anhydrides", Update Nov. 22, 2002

Other

2.4 Acute Aquatic Toxicity to Invertebrates

2.4.1 Acute Aquatic Toxicity to Invertebrates

Test Substance "C1618 ASA: alkenylsuccinic anhydride
CAS # Mixture: 32072-96-1, 28777-98-2, 53520-67-5
Chemical Name Mixture of hexadecenylsuccinic anhydride, octadecenylsuccinic anhydride, eicosenylsuccinic anhydride
Remarks: Purity: 99.82% active ingredient
Method
Method Guideline ASTM Standard E 729-88, "Standard Practice for Conducting

	Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians,
Test Type	Acute Aquatic Toxicity
GLP (Y/N)	Yes
Year	1991
Species/Strain	Saltwater mysid, *Mysidopsis bahia*
Exposure period/duration	96 hours
Analytical monitoring	Yes
Statistical methods	LC50 values and 95% confidence intervals were calculated using the computer program of C.E. Stephan. The program calculated the values using probit analysis, moving average-angle method or binomial probability with nonlinear interpolation. In this study, binomial method was used to evaluate mortality at 48 and 72 hours, and probit method used for 96 hours. The 24 hour LC50 was determined by visual inspection of the mortality data.
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Remarks field for test conditions (fill as applicable)	
Test Species:	Mysids less than 24 hours old obtained from Wildlife International, Ltd. cultures. 20 organisms per test concentration.
Test System:	Each WAF was prepared daily by adding the appropriate amount of test substance to 4 L glass beakers, which were filled with 3 L of salt water, and stirred gently overnight with magnetic stirrers. After stirring, the solutions were allowed to settle for two hours and the water soluble fractions were siphoned from the middle of the beakers and delivered to the test chambers. Test chambers were 500 ml beakers with holes in two sides covered with Teflon screen. The beakers were placed in 2L beakers filled with 1 L of the prepared waters.
Test Conditions:	A static renewal test was conducted in that there was daily renewal of test solution. pH and dissolved oxygen content of the water in treatment and control aquaria were measured at 24 hour intervals before and after renewal.
Light:	Cool-white fluorescent lights provided a light intensity of approximately 60 foot-candles at water surface. Light cycle was controlled to 16 hours of light and 8 hours of darkness..
Test temperature:	Culture waters were 22.4’ to 25.0’ C. Tests were conducted at a target temperature of 25.1’C.
Dilution Water:	Natural seawater collected from Indian River Inlet, Delaware. Salt water was filtered through a sand filter and stored in a 37,800 L tank. Aeration using spray nozzles and filtration (0.2 um) removed organisms and particulates prior to introduction to test system.

	Salinity and pH of negative control water at beginning of test were 24 0/00 and 8.2 respectively.
Test Levels:	Negative Control and nominal concentrations of 8.1, 27, 90, 300 and 1000 mg/L WSF TOC measurements were made at the beginning and at 24 hour intervals to verify concentrations.
Method of calculating mean measured concentrations:	
Exposure period:	96 hours
Analytical monitoring:	Samples of 125 ml were analyzed by a TOC method. Total carbon was determined by a persulfate digestion/infrared detection method on an acidified sample that had been purged of inorganic carbon using nitrogen.
Results	96-h E_{C50} = 169 mg ASA/L 95% confidence limits: 114 and 256 mg ASA/L 96-hr NOEC = 8.1 mg ASA/L
Remarks	
Findings`	All mysids exposed to nominal concentration of 1000 mg ASA/L died during the 24 hour period. Partial mortality was seen at test concentrations of > 27 mg ASA/L. Mysids exposed to 8.1 mg ASA/L showed no signs of treatment related effects. The salt water mysid 96 hour LC50 value for ASA was 169 mg/L, the 95% confidence limits were 114 and 256 mg/L, and the slope of the concentration response curve was 2.0. Based on visual interpretation of the mortality data, the 96 hour no mortality concentration was 8.1 mg ASA/L.
Data Quality	(1) Reliable without restriction
References	D. Murphy, G.T. Peters, "ASA: A 96-Hour Static Renewal Acute Toxicity Test with the Salt Water Mysid (*Mysidopsis Bahia*), Wildlife International, Ltd. Project Number 219A-102A, Sponsored by Ethyl Corporation, 1991.
Other	

2.5 Acute Aquatic Toxicity to Fish

2.5.1 Acute Aquatic Toxicity to Fish

Test Substance	C1618 ASA: alkenylsuccinic anhydride
CAS #	Mixture: 32072-96-1, 28777-98-2, 53520-67-5
Chemical Name	Mixture of hexadecenylsuccinic anhydride, octadecenylsuccinic anhydride, eicosenylsuccinic anhydride
Remarks:	Purity: 99.82% active ingredient
Method	
Method Guideline	ASTM Standard E 729-88, "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians '
Test Type	Acute Aquatic Toxicity
GLP (Y/N)	Yes
Year	1991
Species/Strain	Sheepshead Minnow, *Cyprinodon variegatus*
Exposure period/duration	96 hours
Analytical monitoring	Yes
Statistical methods	The 96 hour LC50 was determined by visual inspection of the mortality data.

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Remarks field for test conditions (fill as applicable)

Test Species:	Juvenile sheepshead minnows were obtained from Wildlife International, Ltd. cultures. All fish were from the same source and year class, and the standard length of the longest fish was no more than twice that of the shortest. Average length of the ten control organisms was 21 mm; average weight of control animals was 0.21 grams. Loading, defined as total wet weight per liter of test solution, was 0.33 grams of fish per liter. Test organisms were acclimated for approximately 52 hours prior to the test. 20 organisms were exposed to each concentration.
Test System:	Each WSF was prepared daily by adding the appropriate amount of test substance to 20 gallon aquaria, which were filled with 15 L of salt water, and stirred gently overnight with magnetic stirrers. After stirring, the solutions were allowed to settle for two hours and the water soluble fractions were siphoned from the middle of the aquaria and delivered to the test chambers. Test chambers were Teflon lined, 25 L polyethylene aquaria filled with 10 L of test solution. The test chambers were randomly positioned in a temperature-controlled environmental chamber designed to maintain a temperature of 22 +/- 1'C.
Test Conditions:	A static renewal test was conducted in that there was daily renewal of test solution. pH and dissolved oxygen content of the water in treatment and control aquaria were measured at 24 hour intervals before and after renewal.

Light:	Fluorescent lights that emitted wavelengths similar to natural sunlight provided a light intensity of approximately 110 foot-candles at water surface. Light cycle was controlled to 16 hours of light and 8 hours of darkness.
Test temperature:	Tests were conducted at a target temperature of 22+/-1'C.
Dilution Water:	Natural seawater collected from Indian River Inlet, Delaware. Salt water was filtered through a sand filter and stored in a 37,800 L tank. Aeration using spray nozzles and filtration (0.2 um) removed organisms and particulates prior to introduction to test system. Salinity and pH of negative control water at beginning of test were 26 0/00 and 8.2 respectively.
Test Levels:	Negative Control and nominal concentrations of 100, 300 and 1000 mg/L WSF TOC measurements were made at the beginning and at 24 hour intervals to verify concentrations.
Method of calculating mean measured concentrations:	not applicable
Exposure period:	96 hours
Analytical monitoring:	Samples of 125 ml were analyzed by a TOC method. Total carbon was determined by a persulfate digestion/infrared detection method on an acidified sample that had been purged of inorganic carbon using nitrogen.
Results	96-hr E_{C50} > 1000 mg ASA/L 96-hr NOEC = 1000 mg ASA/L
Remarks Findings:	All fish exposed to nominal concentration of 1000 mg ASA/L survived for the length of the test and showed no signs of treatment related effects. Based on visual interpretation of the mortality data, the 96 hour no mortality concentration was 1000 mg ASA/L.
Data Quality	(1) Reliable without restriction

References

D. Murphy, G.T. Peters, "ASA: A 96-Hour Static Renewal Acute Toxicity Test with the Sheepshead Minnow *(Cyprinodon variegatus)*, Wildlife International, Ltd. Project Number 219A-101, Sponsored by Ethyl Corporation, 1991.

Other

3.0 Toxicity Category: Alkenyl Succinic Anhydride

3.1 Acute Toxicity

3.1.1 Acute Oral Toxicity

3.1.1.1 Acute Oral Toxicity

Test Substance	***C***12 ASA
CAS #	CAS# 25377-73-5
Chemical Name	Succinic anhydride,dodecenyl-
Remarks	Test material purity not provided.
Method	
Method/Guideline Followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1978
Species/Strain	Rats/ Sherman-Wistar
Sex	Male
No. of animals/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	1, 2, 4, 8 and 16 g/kg
Dose volume	Not Provided
Vehicle control group:	None
Chemical analysis of dosing solution	No
Remarks field for test conditions	(Note: This study was conducted several years prior to the establishment of this test guideline. This report provides a summary of study findings. Individual data are not presented. Single administration of the test material was given intragastrically to five fasted male rats at each dose level. The animals were observed for signs of toxicity or behavioral changes on the day of treatment and throughout the 14-day observation period. Individual weights were recorded immediately prior to dosing and prior to termination. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals.)
Results	LD50 =2.9 (2-4) g/kg (males)
Remarks	During the first three days of study all animals treated at the 4, 8 and 16 g/kg dose levels died. No deaths were observed at the 1 and 2 g/kg dose levels. No clinical signs of toxicity were observed at 1 g/kg. At 2 g/kg the animals were lethargic and had an oily appearance for up to 48 hours post dosing. All animals at the 4, 8 and 16 g/kg dose levels were severely depressed prior to death. No body weight effects occurred at 1 or 2 g/kg. Body weight data was not available at higher dose levels due to the observed mortality. No test material related macroscopic findings were evident.
Conclusions	The test article, when administered to 5 male rats/dose group, had an acute oral LD50 of 2.9 g/kg.

Data Quality	(1) Reliable with restriction Restriction due to the fact that this is a summary report..
References	
Other	Summary taken from ACC HERTG panel Robust Summary "Alkenyl Succinic Anhydrides", Update Nov. 22, 2002

3.1.1.2 Acute Oral Toxicity

Test Substance	$C_{16\text{-}18}$ ***ASA***
CAS#	Mixture: 32072-96-1, 28777-98-2, 53520-67-5
Chemical Name	Mixture of hexadecenylsuccinic anhydride, octadecenylsuccinic anhydride, eicosenylsuccinic anhydride
Remarks	Test material purity: 98% as ASAs
Method	
Method/Guideline Followed	
Test Type	Acute oral toxicity, Limit test
GLP (Y/N)	Y
Year (Study Performed)	1985
Species/Strain	Rats/ Sprague Dawley
Sex	Male and Female
No. of animals/dose	5 male, 5 female
Vehicle	None, administered as received
Route of administration	Oral by gavage
Dose level	5 grams/kg
Dose volume	5 ml/kg
Vehicle control group:	None
Chemical analysis of dosing solution	No
Remarks field for test conditions	Single administration of the test material was given by gavage to five fasted male and five female rats at each dose level. The animals were observed for signs of toxicity or behavioral changes on the day of treatment and throughout the 14-day observation period. Individual weights were recorded immediately prior to dosing and prior to termination. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals.
Results	LD50 > 5 g/kg (males and females)
Remarks	No deaths were observed at the 5 g/kg dose level. Signs observed included diarrhea and wetness of the anogenital region. Body weights increased over the 14 day recovery period.. No test material related macroscopic findings were evident.

Conclusions The test article, when administered to 5 male rats/dose group, had an acute oral LD50 of > 5 g/kg.

Data Quality (1) Reliable without restriction

References V. T. Mallory, "Acute Oral Toxicity Study in Rats (14 day), PH 402-ET-008-84, C16-18 ASA, Lot # Type III", Pharmakon Research International, Inc., sponsored by Ethyl Corporation, 1985.

Other

3.2 Acute Dermal Toxicity

3.2.1 Acute Dermal Toxicity

Test Substance	C_{16-18} ASA
CAS#	Mixture: 32072-96-1, 28777-98-2, 53520-67-5
Chemical Name	Mixture of hexadecenylsuccinic anhydride, octadecenylsuccinic anhydride, eicosenylsuccinic anhydride
Remarks	Test material purity 98% for ASAs
Method	
Method/Guideline Followed	
Test Type	Acute dermal toxicity, Limit test
GLP (Y/N)	Y
Year (Study Performed)	1985
Species/Strain	Rabbits/ Albino New Zealand White
Sex	Male and Female
No.of animals/dose	5 male, 5 female
Vehicle	None, administered as received
Route of administration	Dermal, to clipped, abraded skin site, occluded with gauze, rubber dam
Dose level	5 grams/kg
Dose volume	N/A
Vehicle control group:	None
Chemical analysis of dosing solution	No

Remarks field for test conditions

Single administration of the test material was applied dermally to five male and five female rabbits at each dose level. Sites were abraded before application of test article, and then occluded with gauze, a rubber dam, and ace bandage. After 24 hours of application, the occlusion was removed, and the test sites washed. The animals were observed for signs of toxicity or behavioral changes at 2 and 4 hours on the day of treatment and throughout the 14-day observation period. Individual weights were recorded immediately prior to dosing and prior to termination. The surviving animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals.

Results	LD50 > 5 g/kg (males and females)
Remarks	No deaths were observed at the 5 g/kg dose level. Signs observed included slight to moderate erythema and edema and fissuring of the skin at the site of application. Mean body weights increased for males and stayed the same for females over the 14 day recovery period.. At terminal necropsy, white foci were observed on all lobes of the liver of one animal.
Conclusions	The test article, when administered to 5 male and five female rabbits/ dose group, had an acute oral LD50 of > 5 g/kg.
Data Quality	Reliable without restriction.
References	V.T. Mallory, "Acute Dermal Toxicity Study in Rabbits. PH 422-ET-009-84, C16-18 ASA, Lot # Type III", Pharmakon Research International, Inc., sponsored by Ethyl Corporation, 1985.
Other	

3.3 Acute Inhalation Toxicity

3.3.1 Acute Inhalation Toxicity

Test Substance	3-(dodecenyl) dihydro-2,5 furandione
CAS #	CAS# 25377-73-5
Chemical Name	Succinic anhydride,dodecenyl-
Remarks	Test material purity not provided.
Method	
Method/Guideline Followed	
Test Type	Acute inhalation toxicity, limit test
GLP (Y/N)	Not known
Year (Study Performed)	1982
Species/Strain	Rats/ Sprague Dawley
Sex	5 Male, 5 Female
No. of animals/dose	5
Vehicle	None
Route of administration	Inhalation
Dose level (concentration)	5.3 mg/l nominal 1.22 mg/l calculated
Duration of exposure	4 hours
Vehicle control group:	None
Chemical analysis of dosing solution	No
Remarks field for test Conditions	Two male and two female rats died when exposed for 4 hours to a nominal concentration of 5.3 mg/l C12 ASA. Thus, LC50 for 4 hour exposure is greater than 1.2 mg/l calculated. Clinical signs observed included labored breathing, transient urinary incontinence, alopecia, eye irritation, and body weight loss. No treatment related alterations were seen at gross necropsy.

Results	***4 hour*** LC50	> 1.22 mg/l

Remarks

Conclusions The test article, when administered to rats for 4 hours by inhalation had an LC50 > 1.22 mg/l

Data Quality (1) Reliable with restriction. Restriction due to the fact that this is a summary report.

References Information taken from EPA/OTS Document Number 888100369, TSCA Sect. 8E, recorded 4/19/82, study conducted by Food and Drug Research Laboratories, for Buffalo Color Corporation.

3.4 Primary Dermal Irritation

3.4.1 Primary Dermal Irritation

Test Substance	***$C_{16\text{-}18}$ ASA***
CAS#	Mixture: 32072-96-1, 28777-98-2, 53520-67-5
Chemical Name	Mixture of hexadecenylsuccinic anhydride, octadecenylsuccinic anhydride, eicosenylsuccinic anhydride
Remarks	Test material purity: 98% for ASAs
Method	
Method/Guideline Followed	Draize, 1959, and FHSA 16 CFR 1500.
Test Type	Primary Dermal Irritation
GLP (Y/N)	Y
Year (Study Performed)	1985
Species/Strain	Rabbits/Albino New Zealand White
Sex	Male and Female
No.of animals/dose	3 male, 3 female
Vehicle	None, administered as received
Route of administration	Dermal, to clipped, abraded or non-abraded skin site, occluded with gauze, rubber dam
Dose level	0.5 ml/site
Dose volume	0.5 ml/site
Contact time	24 hours
Vehicle control group:	None
Chemical analysis of dosing solution	No

Remarks field for test conditions

Single administration of the test material was applied dermally to three male and three female rabbits at each dose level. Four sites per animal were prepared by clipping the hair. Two sites were abraded before application of test article; two sites were left intact. Sites were then occluded with gauze, a rubber dam, and ace bandage. After 24 hours of application, the occlusion was removed,

	and the test sites wiped. The animals were observed for signs of erythema and edema and scored according to the Draize scale at 24 and 72 hours after application of treatment and on days 4 and 7. Individual weights were recorded immediately prior to dosing and prior to termination. The surviving animals were euthanized at the conclusion of the observation period
Results	Primary Irritation Index = 2.65
Remarks	No deaths were observed in the test. Signs observed included slight to moderate erythema at 24 and 48 hours, and on day 4. Slight edema of the skin at the site of application was seen at 24 and 72 hours and persisted through day 6. Mean erythema and edema scores were 1.83 and 1.33; and 1.16 and 1.0 for 24 and 72 hours respectively. On day 7, all scores returned to normal and the study was terminated. Mean body weights increased over the course of the study. .
Conclusions	The test article, when administered to 3 male and 3 female rabbits/dose group for 24 hours, caused a primary irritation index of 2.65, which was interpreted as a dermal irritant.
Data Quality	(1) Reliable without restriction

References

V. T. Mallory, "Primary Dermal Irritation Study in Rabbits. PH 420-ET-011-84, C16-18 ASA, Lot # Type III", Pharmakon Research International, Inc., sponsored by Ethyl Corporation, 1985.

Other

3.5 Acute Eye Irritation

3.5.1 Acute Eye Irritation

Test Substance	C_{16-18} ASA
CAS#	Mixture: 32072-96-1, 28777-98-2, 53520-67-5
Chemical Name	Mixture of hexadecenylsuccinic anhydride, octadecenylsuccinic anhydride, eicosenylsuccinic anhydride
Remarks	Test material purity 98% for ASAs
Method	
Method/Guideline Followed	Draize, 1959, and FHSA 16 CFR 1500.
Test Type	Acute Eye Irritation
GLP (Y/N)	Y
Year (Study Performed)	1985
Species/Strain	Rabbits/Albino New Zealand White
Sex	Male and Female
No. of animals/dose	3 male, 3 female
Vehicle	None, administered as received
Route of administration	Directly into eye, no washout

Dose volume	0.1 ml
Vehicle control group:	None
Chemical analysis of dosing solution	No

Remarks field for test conditions

Single administration of the test material was applied into the right eye of three male and three female rabbits. Eyes were examined at 1, 24, 48, and 72 hours and 7 days after treatment. The treated eyes were observed for signs of erythema and edema of the conjunctiva, eversion of the eyelids, ulceraton of the cornea or stippling and opacity, and inflammation of the iris. Grading of the irritation was according to the method of Draize (1965). Classification would be considered as non-irritant if 0 or 1 rabbit had "positive scores" at any time point, and irritant if 4 to 6 animals had positive scores. Individual weights were recorded immediately prior to dosing and prior to termination. The surviving animals were euthanized at the conclusion of the observation period

Results ***Classification was considered irritant***

Remarks

No deaths were observed in the test. Positive ocular scores (for iris, values of "1") were seen at the one hour observation. Scores returned to normal, and the study was terminated on day 7. Mean iris score at 1 hour was 0.87 (some folding). Mean conjunctival score was 1.0 at one hour (some swelling). At 24 hours, all animals had "0" scores for cornea, iris, and conjunctiva except one animal had a value of "1" for edema. All scores for 48 hours and beyond were "0" for all endpoints. Mean body weights increased over the course of the study.

Conclusions

The test article, when administered ocularly to 3 male and 3 female rabbits/dose group, produced threshold values for iris at one hour, which was interpreted an eye irritant under the conditions of the test. In many classification systems, these observations would be interpreted as nonirritant.

Data Quality

(1) Reliable without restriction

References

V. T. Mallory, "Acute Eye Irritation Study in Rabbits. PH 421-ET-008-84, C16-18 ASA, Lot # Type III", Pharmakon Research International, Inc., sponsored by Ethyl Corporation, 1985.

Other

3.6 Skin Sensitization

3.6.1 Skin Sensitization

Test Substance	$C_{16\text{-}18}$ ASA
CAS#	Mixture: 32072-96-1, 28777-98-2, 53520-67-5
Chemical Name	Mixture of hexadecenylsuccinic anhydride, octadecenylsuccinic anhydride, eicosenylsuccinic anhydride
Remarks	Test material purity: 98% for ASAs
Method	
Method/Guideline Followed	Ritz and Buehler, 1980
Test Type	Delayed contact sensitization -Guinea Pigs
GLP (Y/N)	Y
Year (Study Performed)	1987
Species/Strain	Guinea pigs/ Hartley albino
Sex	Male and Female
No. of animals/dose	4 male, 4 female for irritation study 10 male, 10 female for test article 5 male, 5 female for two naïve control groups
Vehicle	Acetone
Route of administration	Dermal for irritation, induction and challenge
Dose level	Irritation test: Undiluted, 50%, 25%, 10%, 5%, 2.5%, 1.0% and 0.5% formulations in acetone Induction: 50% w/v in acetone Primary challenge: 10% w/v in acetone Rechallenge: 3% w/v in acetone
Dose volume	0.3 ml/site
Contact time	6 hours, under Hill Top Chamber
Vehicle control group:	No
Chemical analysis of dosing solution	No

Remarks field for test conditions

In the irritation test, a single administration of the test material was applied dermally to sites clipped of hair on four male and four female guinea pigs. Each animal had 4 sites exposed to a different concentration of test article on a gauze patch. Sites were covered with a Hill Top chamber, which was covered with tape and a rubber dam. After 6 hours of application, the occlusion was removed, and the animals returned to their cages. On the day following application, the clipped sites were depilated with chemical hair remover, and the sites scored for severity of response at 24 hours and 48 hours. The animals were observed for signs of erythema and edema and scored either 0 (no reaction), +/- (slight patchy erythema), 1 (slight confluent or moderate patchy edema), 2 (moderate erythema), and 3 (severe erythema with or without edema).

For induction of sensitization, the upper left quadrant of the backs of guinea pigs were clipped of hair. On the following day,

moistened patches were applied, to the test group, the animals restrained as previously described, and the animals returned to their cages. Patches moistened with test article were applied to the skin in the same manner once a week for three applications. The same site was clipped on the day before application, and the restraint periods were six hours on each occasion.

For primary challenge two weeks after the last of the induction applications, a fresh application site was prepared by clipping the lower left quadrant of the backs of test and naïve control animals. The next day, a challenge patch was applied to each guinea pig in the test and control groups. Each animal was restrained for 6 hours as before, and the animals returned to their cages. On the next day, sites were depilated and scored for severity of response at least two hours later and for a 48 hour reading. Scores of "1" or greater in the test group were considered to be indicative of sensitization providing grades of less than 1 were found in the naïve control group. If the naïve group had grade`1, scores were considered positive in the test animals if greater than the control group scores. In this test, no control animal had a score of "1" in the challenge or rechallenge phase. Seven test animals had a score of "1" at challenge; 9 animals had a score of "1" or greater on rechallenge. The incidence and severity of responses were more pronounced in the test group indicating that a sensitization response had been elicited.

Conclusions The test article, when administered to guinea pigs according to the method of Ritz and Buehler, caused delayed contact hypersensitivity of the skin.

Data Quality (1) Reliable without restriction

References: Ritz, H.L, and Buehler, E.V, (1980), In Current Concepts in Cutaneous Toxicity (V.A. Drill and T. Lazar, eds.) pp. 25-40, Academic Press, New York.

Other: Buehler, E.V., "Delayed Contact Hypersensitivity Study in Guinea Pigs of: ASA Alkenylsuccinic Anhydride" for Ethyl Corporation, Hill Top Research Project No. 86-0873-21, Hill Top Research, Inc., 1986.

3.6.2 Skin Sensitization

Test Substance	Dodecenyl Succinic Anhydride
CAS#	CAS #27859-58-1
Chemical Name	Dodecenyl Succinic Anhydride (C12 ASA)
Remarks	Test material purity: 98% for ASAs
Method	
Method/Guideline Followed	Ritz and Buehler, 1980
Test Type	Delayed contact sensitization -Guinea Pigs
GLP (Y/N)	Y

Year (Study Performed)	1986
Species/Strain	Guinea pigs/ Hartley albino
Sex	Male and Female
No.of animals/dose	4 male, 4 female for irritation study 10 male, 10 female for test article 5 male, 5 female for two naïve control groups
Vehicle	Acetone
Route of administration	Dermal for irritation, induction and challenge
Dose level	Irritation test: Undiluted, 50%, 25%, 10%, 5%, 2.5%, and 1.0% formulations in acetone Induction: 25% w/v in acetone Primary challenge: 5% w/v in acetone Rechallenge: 3 % w/v in acetone
Dose volume	0.3 ml/site
Contact time	6 hours, under Hill Top Chamber
Vehicle control group:	No
Chemical analysis of dosing solution	No

Remarks field for test conditions

In the irritation test, a single administration of the test material was applied dermally to sites clipped of hair on four male and four female guinea pigs. Each animal had 4 sites exposed to a different concentration of test article on a gauze patch. Sites were covered with a Hill Top chamber, which was covered with tape and a rubber dam. After 6 hours of application, the occlusion was removed, and the animals returned to their cages. On the day following application, the clipped sites were depilated with chemical hair remover, and the sites scored for severity of response at 24 hours and 48 hours. The animals were observed for signs of erythema and edema and scored either 0 (no reaction), +/- (slight patchy erythema), 1 (slight confluent or moderate patchy edema), 2 (moderate erythema), and 3 (severe erythema with or without edema).

For induction of sensitization, the upper left quadrant of the backs of guinea pigs were clipped of hair. On the following day, moistened patches were applied, to the test group, the animals restrained as previously described, and the animals returned to their cages. Patches moistened with test article were applied to the skin in the same manner once a week for three applications. The same site was clipped on the day before application, and the restraint periods were six hours on each occasion.

For primary challenge two weeks after the last of the induction applications, a fresh application site was prepared by clipping the lower left quadrant of the backs of test and naïve control animals. The next day, challenge patch was applied to each guinea pig in the test and control groups. Each animal was restrained for 6 hours as

before, and the animals returned to their cages. On the next day, sites were depilated and scored for severity of response at least two hours later and for a 48 hour reading. Scores of "1" or greater in the test group were considered to be indicative of sensitization providing grades of less than 1 were found in the naïve control group. If the naïve group had grades of "1" or greater, the reactions in the test group that exceeded the most severe reactions in the control group were presumed to be indicative of sensitization.

For rechallenge, eight days after the primary challenge, all of the original animals were single patch rechallenged. Ten previously unexposed naïve animals were identically treated to serve as a new naïve control group. The right rear quadrant was used for rechallenge. Depilation and observation procedures were the same as described for primary challenge.

Individual weights were recorded immediately prior to dosing and prior to termination. The surviving animals were euthanized at the conclusion of the observation period

Results No deaths were observed in the test. The incidence of grade "2" responses or greater in the test group exposed to 25% for induction, and 5% at primary challenge was greater for the test animals (13 of 20) compared to the naïve challenge group (0 of 10). The incidence and severity of responses in the test group were more pronounced than the responses of the naïve group, suggesting sensitization had occurred.

At rechallenge with 3% test article in acetone, the incidence of grade "1" responses or greater in the test group (17 of 20) was greater than that in the naïve control group (0 of 10). The incidence and severity of responses were more pronounced in the test group indicating that a sensitization response had been elicited.

Conclusions The test article, when administered to guinea pigs according to the method of Ritz and Buehler, caused delayed contact hypersensitivity of the skin.

Data Quality (1) Reliable without restriction

References: Ritz, H.L, and Buehler, E.V., (1980), In Current Concepts in Cutaneous Toxicity, (V.A. Drill and T.Lazar, eds.) pp. 25-40, Academic Press, New York.

Other: Buehler, E.V., "Delayed Contact Hypersensitivity Study in Guinea Pigs of: DODECENYL SUCCINIC ANHYDRIDE C12 ASA" for Ethyl Corporation, Hill Top Research Project No. 86-0873-21, Hill Top Research, Inc., 1986.

3.6.3 Skin Sensitization

Test Substance $C_{16\text{-}18}$ ASA

CAS# Mixture: 32072-96-1, 28777-98-2, 53520-67-5

Chemical Name	Mixture of hexadecenylsuccinic anhydride, octadecenylsuccinic anhydride, eicosenylsuccinic anhydride
Remarks	Test material purity: 98% for ASAs
Method	
Method/Guideline Followed	Magnusson and Kligman, 1969
Test Type	Guinea Pig Sensitization Maximization Test
GLP (Y/N)	Y
Year (Study Performed)	1985
Species/Strain	Guinea pigs/ Hartley albino
Sex	Male and Female
No.of animals/dose	4 male, 4 female for irritation study 10 male, 10 female for test article 3 male, 3 female for positive control 2 male, 2 female for vehicle control
Vehicle	0.9% saline for intradermal injection 80% ethanol for topical applications
Route of administration	Intradermal for first induction Dermal for irritation, second induction, challenge and rechallenge
Dose level, test article	Irritation test: Undiluted, 50%, 25%, 10%, 5%, 4%, 3%, 2%, and 1.0% formulations Intradermal rangefinder: 5%, 4%, 3%, 2%, 1% Intradermal induction: 1% Topical induction: 5% Primary challenge: 1% Rechallenge: 0.5%
Dose level, positive control	DNCB 0.1% for intradermal induction DNCB 0.1% topical and challenge
Dose volume	0.1 ml/site for intradermal injection
Frequency of administration	Once each for ID and topical induction Once each for topical challenge and rechallenge
Vehicle control group:	Yes
Chemical analysis of dosing solution	No
Remarks field for test conditions	In the intradermal rangefinder test, a single administration of the test material was applied intradermally to sites clipped of hair on two male and two female guinea pigs. Each animal had 6 sites

exposed to a different concentration of test article. The animals were observed for signs of erythema and edema and were scored at 24 hours as either 0 (no reaction), +/- (slight patchy erythema), 1 (slight confluent or moderate patchy edema), 2 (moderate erythema), and 3 (severe erythema with or without edema). Based on results, the dose chosen for intradermal injection was 1.0%.

For the dermal range finding study, eight unexposed animals were topically induced with different concentrations of test article. Skin on the sides was shaved of hair, test article applied, and sites wrapped for 24 hours. Readings were made at 24 hours after unwrapping and followed the above described scoring. The dose chosen for topical induction was 5%. Challenge dose was chosen to be 1% and rechallenge dose 0.5%.

For intradermal induction of sensitization, the shoulders of guinea pigs were clipped of hair. All intradermal injections for these groups (test article group, vehicle group, and positive control groups) were given in this shoulder area. Three injections were given in each site: 1) 0.1 ml of Freund's Complete Adjuvant 2) 0.1 ml of test article, vehicle or positive control and 3) 0.1 ml test article, vehicle or positive control mixed with Freund's complete adjuvant.

Topical induction was conducted seven days after intradermal induction. The sites were clipped of hair. Filter paper (2 x 4 cm) was saturated with experimental material, vehicle or positive control substance and applied to the injection site area and occluded with a rubber dam under a bandage. Bandaging was removed after 48 hours

For primary challenge two weeks after the last of the induction applications, a fresh application site was prepared by clipping the left and right flanks of test and naïve control animals. Challenge patches were applied to each guinea pig in the test and control groups under occlusion. Twenty four hours later, the sites were wiped clean and clipped of hair. Three hours later, sites were scored for severity of response and again 24 hours later. Kligman's classification scheme modified to reflect a treatment group of twenty animals was used to rank the substances in order of their sensitization capacity. According to the percentage of animals sensitized, the substance was assigned to one of five classes ranging from weak (0-8%, grade 1) to extreme (81-100%, grade V) regardless of the intensity of the response. Magnusson and Kligman do not consider sensitization grade 1 as significant.

For rechallenge, six days after the primary challenge, all of the original animals were single patch rechallenged.

Individual weights were recorded immediately prior to dosing and prior to termination. The surviving animals were euthanized at the conclusion of the observation period

Results No deaths were observed in the test. The positive control group (DNCB) showed a positive sensitization response. The treated group (C1618 ASA, 1%) produced a 35% positive response corresponding to sensitization grade III (moderate, 29-64%). Rechallenge with 0.5%ASA caused positive responses in 15% of the test animals corresponding to a sensitization grade II (mild, 9-28%).

Conclusions The test article, when administered to guinea pigs according to the method of Magnusson and Kligman, caused sensitization of the skin.

Data Quality (1) Reliable without restriction

V.T. Mallory, "Guinea Pig Sensitization Maximization Test (Magnusson-Kligman) C1618 ASA" PH 423-ET-001-84: Pharmakon Research International, Inc., sponsored by Ethyl Corporation, 1986.

4.0 MUTAGENICITY:

4.1 Bacterial Mutagenicity

Test Substance	$C_{16\text{-}18}$ ASA
CAS#	Mixture: 32072-96-1, 28777-98-2, 53520-67-5
Chemical Name	Mixture of hexadecenylsuccinic anhydride, octadecenylsuccinic anhydride, eicosenylsuccinic anhydride
Remarks	Test material purity 98% for ASAs
Method	
Method/Guideline Followed	Revised methods for the Salmonella Mutagenicity Test, Maron, DM and B.N Ames, 1983
Test Type	Bacterial Mutagenicity: Plate Incorporation Assay
GLP (Y/N)	Y
Year (Study Performed)	1985
Species/Strain	Salmonella typhimurium, TA 1535, TA 1537, TA1538, TA 98, TA 100
Source:	Dr. Bruce Ames, University. of California, Berkeley, California
Vehicle	Acetone
Positive Controls:	Sodium Azide: TA 1535, TA 100, without activation 10 ug/plate 9-aminoacridine: TA 1537 without activation

	150 ug/plate 2-nitrofluorene: TA 98, TA 1538 without activation 5 ug/plate 2-aminoanthracene: all strains with activation 5.0 ug/plate
Dose levels:	0.5, 1.6, 5.0, 16 and 50 ug ASA/plate
Vehicle control group:	
Chemical analysis of dosing solution	No
Remarks field for test conditions	
Test organism preparation	Frozen stock cultures were prepared from frozen master cultures and, after 10-12 hours growth period, aliquoted in 1 ml culture media into Nunc vials, and quick frozen before being stored at a minimum of –60'C. Fresh cultures were prepared by thawing a vial of frozen working stock cultures of each tester strain and transferring the culture to 25 ml Oxoid Nutrient Broth #2, and grown for approximately 10 hours at 37'C in an incubator/shaker. After incubation, samples were diluted 1:4 in distilled water and optical densities observed. Historical data has shown that optical densities of 0.4 are representative of cells in late exponential or early stationary phase of growth. Tester strains were checked monthly for appropriate genetic markers.
Negative and Positive controls:	Tester strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 were plated in triplicate with the appropriate solvent, both with and without metabolic activation to obtain background lawn and revertant colony formation to serve as negative solvent controls. All tester strains were also run in triplicate with known positive response chemicals.
Top Agar:	Used as an overlay was reconstituted into a molten state, and supplemented with 0.5 mM histidine and 0.5 mM biotin at a volume of 0.1 ml per ml of agar, and maintained at 45'C until use. All negative and positive tubes and control plates, and all compound treated plates, and all compound treated tubes and plates were prepared in triplicate. Tubes were prepared with 2 ml aliquots of top agar, 0.1 ml of tester strain, and 0.1 ml of the appropriate concentration of test compound. The tubes were vortexed, and the contents poured onto minimal glucose plates. The sample was evenly distributed on the plate and the top agar overlay allowed to harden.
Metabolic Activation System:	S-9 fraction of rat liver homogenate from Aroclor 1254-treated Sprague Dawley rats. S-9 fraction was thawed on the day of use and 0.5 ml of S-9 mix added to tubes which

	required metabolic activation, in addition to the preceding top agar ingredients. Tubes were then vortexed and poured on minimal glucose plates. Plates were allowed to harden.
Process:	Within an hour of plating, plates were inverted and placed in a dark 37'C incubator. Plates were incubated for 48-72 hours, checked for uniform background lawn, and scored by counting revertant colonies on an electronic colony counter interfaced with a computer for data acquisition.
Results:	There were no observed increases in mutation frequencies in strains TA 1535, TA 1537, TA 1538, TA 98, and TA100 of Salmonella typhimurium both with and without metabolic activation at doses of 0.5, 1.6, 5.0, 16, and 50 ug/plate. All solvent and positive controls were within the acceptable limits of mean historic data.
Conclusion:	The test article was negative for mutagenicity within the conditions of this test in strains TA 1535, TA 1537, TA 1538, TA 98, and TA100 of Salmonella typhimurium both with and without metabolic activation at doses of 0.5, 1.6, 5.0, 16, and 50 ug/plate.

Data Quality (1) Reliable without restriction

References

T.R. Barfknecht, "Ames Salmonella/Microsome Plate Test (EPA/OECD). PH 301-ET-004-84, ASA, Lot # Type III", Pharmakon Research International, Inc., sponsored by Ethyl Corporation, 1985.

Other

4.2 In vitro Mammalian Cell Mutagenicity

4.2.1 Unscheduled DNA Synthesis

Test Substance	$C_{16\text{-}18}$ ASA
CAS#	Mixture: 32072-96-1, 28777-98-2, 53520-67-5
Chemical Name	Mixture of hexadecenylsuccinic anhydride, octadecenylsuccinic anhydride, eicosenylsuccinic anhydride
Remarks	Test material purity 98% for ASAs
Method	
Method/Guideline Followed	Revised method of Williams, 1978
Test Type	Rat Hepatocyte Primary Culture/DNA Repair Test
GLP (Y/N)	Y
Year (Study Performed)	1985

Species/Strain	Rat/ Fischer-344
Vehicle	DMSO
Positive Controls:	2-acetamidofluorene at 10^{-6} M
Dose levels evaluated :	5, 20 and 50 ug ASA/well
Vehicle control group:	Yes
Chemical analysis of dosing solution	No
Remarks field for test conditions	
Hepatocyte preparation	Male Fisher rats were anesthetized with sodium Nembutal by intraperitoneal injection. The livers were exposed surgically, perfused, and removed. The livers were excised, and isolated hepatocytes prepared. Freshly isolated hepatocytes were treated with 20 ul of ASA at 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500, and 1000 ug/well in 2 mL of media.
Negative and Positive controls:	An acetone group, an untreated control, and a 2AAF (2-acetamidofluorene) group were evaluated concurrently with the treatment groups.
Process:	Hepatocytes were treated with test article, fixed on microscope cover slides, stained, dipped, and developed. Unscheduled DNA repair synthesis, evidenced by a net increase in black silver grains in the nucleus, was quantified by determining nuclear and background grain counts for 25 cells per slide, or as many cells as possible up to 25 in the presence of toxicity. An automatic colony counter with a microscope attachment was used for the counts. This value was determined by taking a nuclear count and the average of three adjacent cytoplasmic counts. A positive test would be based on production of a mean grain count of five or greater than the vehicle control mean grain count and a statistically significant difference between test article treated cells and the vehicle control in the number of cells with net nuclear grain counts greater than zero.
Results:	Cytotoxicity was produced at 100, 500 and1000 ug/well. Test article did not cause an increase in mean net nuclear counts over the acetone control treated cells at any dose level counted (50, 10 and 5 ug/well. All solvent and positive controls were within the acceptable limits of mean historic data.

Conclusion:	The test article was negative for mutagenicity within the conditions of this test. ASA was not able to produce a mean grain count of five or greater than the vehicle control mean grain count, and no statistical difference between the ASA treated cells and vehicle control in number of cells with a net nuclear grain count greater than zero was produced. A dose response increase in net nuclear counts or cells greater than zero was not demonstrated for ASA.
Data Quality	Reliable without restriction
References	D. E. Johnson, "Genetic Toxicology Rat Hepatocyte Primary Culture/DNA Repair Test," C16-18 alkenyl succinic anhydride, ASA, ", Ethyl Corporation Technical Center. 1984.
Other	